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APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: PLANT TUBBY-LIKE PROTEINS

APPLICANT: JEI-FU SHAW AND CHIA-PING LAI

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PLANT TUBBY-LIKE PROTEINS

RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 60/441,380, filed on January 21, 2003, the contents of which are incorporated by reference in their entirety.

BACKGROUND

Various environmental factors, e.g., high salinity, pathogens, and chilling, cause stress and adverse effects on growth and productivity of crops. It is therefore desirable to produce transgenic crops that are tolerant to such factors. Genetic engineering can be used to modify proteins that are involved in regulating responses of plants to environmental factors, thereby improving stress-tolerance.

TUBBY proteins, a group of membrane-bound transcription regulators, were first identified from obese mice via positional cloning (Kleyn et al., 1996, Cell 85: 281-290 and Noben-Trauth et al., 1996, Nature 380: 534-538.). Mutations in the TUBBY genes lead to maturity-onset obesity, insulin resistance, retinal degeneration, and neurosensory hearing loss. TUBBY-like proteins (TLPs) were subsequently discovered in other mammals and were found to be activated through G-proteins, which, in higher plants, are involved in the response to environmental factors and hormone regulation (Warpeha et al., 1991, Proc. Natl. Acad. Sci. 88: 8925-8929, and Ueguchi-Tanaka et al., Proc. Natl. Acad. Sci. 97: 11638-11643).

SUMMARY

This invention is based on the discovery of eleven *Arabidopsis* TUBBY-like proteins, designated as AtTLPs 1-11. These proteins regulate the response of *Arabidopsis* to environmental factors. The full-length AtTLPs 1-11 polypeptides (designated as SEQ ID NOs: 1-11), and cDNAs encoding the polypeptides (designated as SEQ ID NOs: 12-22), are shown below:

AtTLP1:

polypeptide:

```
  1  MSFRSIVRDV RDSIGSLSRR SFDFKLSSLN KEGGKSRGSV QDSHEEQLVV
 51  TIQETPWANL PPELLRDVIK RLEESESVWP ARRHVVACAS VCRSWRDMCK
101  EIVQSPELSG KITFPVSLKQ PGPRDATMQC FIKRDKSNLT YHLYLCLSPA
151  LLVENGKFLL SAKRIRRTTY TEYVISMHAD TISRSSNTYI GKIRSNFLGT
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201  KFIIYDTQPA YNSNIARAVQ PVGLSRRFYS KRVSPKVPSG SYKIAQVSYE
251  LNVLGTRGPR RMHCAMNSIP ASSLAEGGTV PGQPDIIVPR SILDESFRSI
301  TSSSSRKITY DYSNDFSSAR FSDILGPLSE DQEVVLEEGK ERNSPPLVLK
351  NKPPRWHEQL QCWCLNFRGR VTVASVKNFQ LIAANQPQPQ PQPQPQPQPL
401  TQPQPSGQTD GPDKIILQFG KVGKDMFTMD FRYPLSAFQA FAICLSSFDT
451  KLACE  (SEQ ID NO: 1)
```

cDNA:

```
  1  ATGTCGTTCC GTAGCATAGT TCGTGATGTG AGAGATAGTA TAGGAAGTCT
 51  ATCGAGGCGT AGTTTCGACT TTAAGTTAAG CAGCTTGAAC AAAGAAGGTG
101  GTAAATCCCG TGGTTCGGTT CAAGATTCTC ATGAGGAACA ACTTGTAGTA
151  ACGATTCAAG AAACACCGTG GGCGAATCTA CCTCCAGAGT TATTACGTGA
201  TGTGATCAAA AGACTTGAAG AGAGTGAAAG TGTGTGGCCT GCTCGTAGAC
251  ATGTTGTTGC TTGTGCTTCT GTTTGCAGGT CATGGAGAGA TATGTGTAAA
301  GAGATTGTTC AAAGTCCGGA GCTCTCAGGC AAAATCACAT TTCCTGTTTC
351  GTTGAAACAG CCTGGACCAA GAGATGCAAC AATGCAATGC TTTATCAAAA
401  GGGATAAATC TAACTTGACT TATCATTTAT ATCTTTGTCT CAGTCCTGCT
451  TTGTTGGTTG AGAATGGAAA GTTTCTTCTT TCTGCAAAAC GCATAAGAAG
501  AACTACATAC ACCGAGTACG TGATCTCTAT GCACGCCGAC ACCATTTCGA
551  GATCAAGCAA TACCTACATT GGCAAAATCA GGTCTAATTT TCTGGGGACG
601  AAGTTTATAA TATACGATAC ACAACCAGCA TACAACAGCA ACATCGCTCG
651  AGCGGTCCAA CCGGTAGGTC TTAGCCGCAG ATTCTACTCA AAGAGAGTCT
701  CTCCCAAAGT ACCTAGTGGG AGCTACAAAA TTGCGCAGGT TTCTTATGAG
751  CTAAACGTTC TTGGTACCCG TGGTCCGAGG AGAATGCATT GTGCGATGAA
801  CTCAATTCCC GCCTCTTCCC TTGCGGAAGG CGGAACTGTG CCTGGACAGC
851  CCGATATCAT TGTCCCGCGC TCTATTCTCG ACGAATCGTT CCGCAGCATT
901  ACCTCTTCGT CATCGAGAAA AATCACTTAC GATTACTCGA ATGATTTTAG
951  CAGTGCACGG TTTTCCGACA TTCTTGGCCC GTTAAGCGAA GACCAAGAAG
1001 TGGTATTAGA AGAAGGGAAA GAGCGGAATT CGCCACCACT TGTGCTTAAG
1051 AACAAGCCGC CGAGGTGGCA TGAACAGCTT CAGTGTTGGT GTTTAAACTT
1101 CAGGGGACGT GTAACAGTCG CATCAGTTAA GAACTTTCAG CTCATTGCAG
1151 CAAACCAACC ACAGCCTCAG CCTCAGCCTC AACCGCAACC TCAACCCCTA
1201 ACTCAGCCGC AACCGTCTGG TCAGACCGAT GGTCCCGACA AGATCATATT
1251 GCAGTTTGGG AAAGTGGGAA AAGACATGTT CACGATGGAT TTCCGGTATC
1301 CGCTCTCTGC GTTTCAGGCT TTCGCTATCT GTTTGAGCAG TTTCGACACA
1351 AAACTTGCTT GCGAA   (SEQ ID NO: 12)
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AtTLP2:

polypeptide:

```
  1  MSLKSILRDL KEVRDGLGGI SKRSWSKSSH IAPDQTTPPL DNIPQSPWAS
 51  LPPELLHDII WRVEESETAW PARAAVVSCA SVCKSWRGIT MEIVRIPEQC
101  GKLTFPISLK QPGPRDSPIQ CFIKRNRATA TYILYYGLMP SETENDKLLL
151  AARRIRRATC TDFIISLSAK NFSRSSSTYV GKLRSGFLGT KFTIYDNQTA
201  SSTAQAQPNR RLHPKQAAPK LPTNSSTVGN ITYELNVLRT RGPRRMHCAM
251  DSIPLSSVIA EPSVVQGIEE EVSSSPSPKG ETITTDKEIP DNSPSLRDQP
301  LVLKNKSPRW HEQLQCWCLN FKGRVTVASV KNFQLVAEID ASLDAPPEEH
351  ERVILQFGKI GKDIFTMDYR YPLSAFQAFA ICISSFDTKP ACEG (SEQ ID NO: 2)
```

cDNA:

```
  1  ATGTCTTTGA AAAGCATCCT TCGTGATCTG AAGGAAGTGA GGGATGGACT
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  51  TGGAGGCATC TCCAAGAGAA GCTGGTCAAA GTCGTCTCAC ATTGCTCCTG
 101  ATCAAACAAC TCCACCACTG GATAACATAC CACAGAGCCC ATGGGCTTCT
 151  TTGCCGCCTG AGTTGCTTCA TGACATTATC TGGAGGGTTG AAGAGAGTGA
 201  GACAGCTTGG CCCGCTCGAG CTGCCGTTGT CTCTTGTGCT TCAGTATGTA
 251  AATCATGGAG AGGAATCACT ATGGAGATTG TGAGGATCCC TGAGCAGTGT
 301  GGGAAGCTCA CTTTTCCAAT CTCATTGAAA CAGCCGGGGC CTCGAGACTC
 351  TCCAATTCAA TGTTTTATTA AGAGGAACAG AGCAACAGCT ACATACATTC
 401  TCTATTATGG TTTGATGCCT TCGGAGACTG AGAACGACAA ACTGTTGTTA
 451  GCAGCAAGAA GGATTAGAAG AGCGACATGC ACAGACTTTA TAATCTCCCT
 501  ATCTGCAAAG AACTTCTCAC GGAGCAGCAG TACTTATGTT GGCAAATTAA
 551  GGTCTGGTTT TCTGGGAACC AAGTTCACAA TATATGACAA CCAAACAGCA
 601  TCATCCACAG CACAAGCCCA ACCTAACCGA AGACTCCACC CGAAACAAGC
 651  GGCTCCTAAA CTACCTACGA ATAGCTCTAC CGTAGGAAAC ATAACCTACG
 701  AGCTCAATGT TCTTCGCACA AGGGGACCTA GAAGAATGCA CTGCGCTATG
 751  GATTCTATAC CCCTCTCTTC TGTTATTGCT GAACCGTCAG TAGTTCAAGG
 801  CATAGAAGAG GAAGTCTCTT CCTCTCCTTC ACCAAAAGGA GAAACCATCA
 851  CAACAGACAA AGAGATTCCT GATAATTCTC CAAGCTTAAG GGACCAACCG
 901  CTAGTTCTCA AAAACAAATC CCCAAGATGG CATGAGCAGT TGCAGTGCTG
 951  GTGCCTCAAC TTCAAGGGAA GAGTGACTGT GGCTTCAGTT AAGAATTTCC
1001  AGCTTGTTGC AGAGATTGAC GCTTCTTTGG ATGCGCCGCC TGAAGAACAT
1051  GAGAGGGTGA TCTTACAGTT TGGCAAAATC GGTAAGGATA TTTTCACCAT
1101  GGATTATCGC TACCCTCTAT CTGCTTTTCA AGCCTTTGCT ATATGCATTA
1151  GCAGCTTTGA CACCAAACCG GCATGTGAAG GG (SEQ ID NO: 13)
```

AtTLP3:

polypeptide:

```
   1  MSFKSLIQDM RGELGSISRK GFDVRFGYGR SRSQRVVQDT SVPVDAFKQS
  51  CWASMPPELL RDVLMRIEQS EDTWPSRKNV VSCAGVCRNW REIVKEIVRV
 101  PELSSKLTFP ISLKQPGPRG SLVQCYIMRN RSNQTYYLYL GLNQAASNDD
 151  GKFLLAAKRF RRPTCTDYII SLNCDDVSRG SNTYIGKLRS NFLGTKFTVY
 201  DAQPTNPGTQ VTRTRSSRLL SLKQVSPRIP SGNYPVAHIS YELNVLGSRG
 251  PRRMQCVMDA IPASAVEPGG TAPTQTELVH SNLDSFPSFS FFRSKSIRAE
 301  SLPSGPSSAA QKEGLLVLKN KAPRWHEQLQ CWCLNFNGRV TVASVKNFQL
 351  VAAPENGPAG PEHENVILQF GKVGKDVFTM DYQYPISAFQ AFTICLSSFD
 401  TKIACE (SEQ ID NO: 3)
```

cDNA:

```
   1  ATGTCCTTCA AGAGTCTCAT TCAGGACATG AGAGGAGAGC TTGGGAGTAT
  51  ATCCAGAAAG GGATTCGATG TCAGATTCGG GTATGGTAGA TCCAGGTCTC
 101  AACGTGTTGT TCAGGATACT TCTGTTCCTG TTGATGCTTT CAAGCAGAGC
 151  TGCTGGGCTA GTATGCCTCC GGAGCTCCTG AGAGATGTTC TTATGAGGAT
 201  TGAGCAATCC GAAGACACTT GGCCGTCTAG GAAAAATGTT GTTTCTTGCG
 251  CTGGTGTCTG CAGGAACTGG CGAGAAATCG TCAAAGAGAT CGTCAGAGTT
 301  CCTGAGCTTT CTAGCAAACT CACTTTTCCT ATCTCCCTCA AACAGCCGGG
 351  TCCTAGAGGA TCACTTGTTC AATGCTATAT TATGAGAAAC CGCAGCAATC
 401  AAACCTACTA TCTATACCTC GGGTTAAACC AAGCAGCTTC AAATGATGAT
 451  GGAAAGTTCC TTCTTGCTGC CAAGAGGTTT CGGAGGCCAA CTTGCACTGA
 501  CTACATCATC TCCTTAAACT GCGATGATGT CTCTCGAGGA AGCAATACCT
 551  ATATCGGAAA GCTTAGATCT AACTTTCTGG GGACCAAGTT CACTGTCTAT
 601  GACGCTCAGC CGACGAATCC TGGAACTCAG GTTACCAGAA CCCGTTCAAG
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 651  CAGACTTCTC AGTTTGAAAC AAGTGAGCCC GAGAATTCCA TCTGGCAACT
 701  ATCCTGTAGC ACATATCTCA TATGAGCTTA ACGTCTTGGG TTCCAGAGGA
 751  CCGAGGAGGA TGCAGTGTGT CATGGATGCC ATCCCTGCAT CAGCTGTAGA
 801  ACCTGGAGGA ACAGCTCCAA CTCAGACGGA ACTTGTCCAT AGCAATCTTG
 851  ATAGTTTCCC CTCATTCTCC TTCTTCAGGT CGAAATCAAT TCGTGCAGAG
 901  AGTCTCCCTT CTGGTCCATC ATCTGCTGCT CAGAAGGAAG GACTGCTTGT
 951  CCTGAAAAAC AAAGCGCCCA GATGGCACGA ACAGCTCCAG TGCTGGTGCC
1001  TCAACTTCAA TGGGAGAGTC ACAGTTGCTT CCGTCAAAAA CTTTCAGCTG
1051  GTAGCTGCTC CTGAGAATGG ACCTGCAGGA CCTGAGCACG AAAACGTGAT
1101  TCTCCAGTTT GGAAAAGTCG GAAAAGATGT GTTCACAATG GATTATCAGT
1151  ACCCTATCTC TGCCTTCCAG GCCTTCACCA TTTGCCTCAG CAGTTTCGAC
1201  ACCAAGATAG CATGTGAA  (SEQ ID NO:14)
```

AtTLP4:

polypeptide:

```
   1  MPPELLRDVL MRIERSEDTW PSRKNVVSCV GVCKNWRQIF KEIVNVPEVS
  51  SKFTFPISLK QPGPGGSLVQ CYVKRNRSNQ TFYLYLGGEA KIFCQSEPSD
 101  IYLVPYSYRE THCVMDAISA SAVKPGGTAT TQTELDNFVS FRSPSGQKEG
 151  VLVLKSKVPR LEEQSWCLDF NGSPENEPEN ENDIFQFAKV GNLHKLFSLY
 201  EAEWIPLVRT SVFAVIARVC RDKKHTPSYE LKLALYFAKN SAILKKFVLR
 251  GYTREEDLLA LPVAN  (SEQ ID NO:4)
```

cDNA:

```
   1  ATGCCTCCTG AGCTTCTGAG AGATGTTCTG ATGAGGATAG AGCGATCCGA
  51  AGACACTTGG CCTTCTAGGA AGAATGTTGT TTCTTGTGTA GGTGTGTGTA
 101  AGAACTGGCG ACAAATATTC AAAGAGATCG TTAACGTTCC TGAGGTTTCT
 151  AGCAAATTCA CTTTTCCAAT CTCCTTGAAA CAGCCTGGTC CAGGAGGATC
 201  ACTTGTTCAA TGCTATGTTA AGAGAAACCG TAGCAATCAA ACTTTCTATC
 251  TATACCTTGG AGGTGAAGCA AAAATATTTT GTCAGTCTGA ACCAAGTGAT
 301  ATTTATCTCG TTCCTTACAG TTACAGAGAG ACGCATTGCG TCATGGATGC
 351  CATCTCTGCA TCAGCAGTAA AACCTGGAGG AACAGCTACA ACTCAGACAG
 401  AACTCGATAA TTTCGTGTCA TTCAGGTCTC CTTCTGGTCA AAAGGAAGGA
 451  GTGCTTGTTC TTAAGAGCAA AGTGCCTAGA TTGGAAGAAC AGAGCTGGTG
 501  TCTCGACTTC AATGGCTCTC CTGAGAACGA ACCTGAGAAT GAAAACGACA
 551  TTTTCCAGTT TGCGAAAGTC GGAAACTTGC ACAAACTCTT CAGTTTATAT
 601  GAGGCTGAAT GGATTCCTCT CGTTCGCACC TCAGTGTTTG CTGTCATTGC
 651  TCGAGTTTGT AGAGATAAAA AGCATACACC ATCGTATGAA TTGAAACTTG
 701  CATTGTACTT TGCAAAAAAC TCTGCAATCC TCAAGAAATT CGTTCTCCGC
 751  GGTTACACTC GAGAAGAAGA TTTACTCGCA TTGCCCGTGG CTAAC  (SEQ ID NO:15)
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AtTLP5:

polypeptide:

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   1  MSFLSIVRDV RDTVGSFSRR SFDVRVSNGT THQRSKSHGV EAHIEDLIVI
  51  KNTRWANLPA ALLRDVMKKL DESESTWPAR KQVVACAGVC KTWRLMCKDI
 101  VKSPEFSGKL TFPVSLKQPG PRDGIIQCYI KRDKSNMTYH LYLSLSPAIL
 151  VESGKFLLSA KRSRRATYTE YVISMDADNI SRSSSTYIGK LKSNFLGTKF
 201  IVYDTAPAYN SSQILSPPNR SRSFNSKKVS PKVPSGSYNI AQVTYELNLL
 251  GTRGPRRMNC IMHSIPSLAL EPGGTVPSQP EFLQRSLDES FRSIGSSKIV
 301  NHSGDFTRPK EEEGKVRPLV LKTKPPRWLQ PLRCWCLNFK GRVTVASVKN
 351  FQLMSAATVQ PGSGSDGGAL ATRPSLSPQQ PEQSNHDKII LHFGKVGKDM
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401 FTMDYRYPLS AFQAFAISLS TFDTKLACE (SEQ ID NO: 5)

cDNA:

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   1 ATGTCGTTTC TGAGTATTGT TCGTGATGTT AGAGATACTG TAGGAAGCTT
  51 TTCGAGACGT AGTTTCGACG TGAGAGTATC TAATGGGACG ACTCATCAGA
 101 GGAGTAAATC TCACGGTGTT GAGGCACATA TTGAAGATCT TATTGTAATC
 151 AAGAACACTC GTTGGGCTAA TTTACCGGCT GCGCTATTAC GAGATGTGAT
 201 GAAAAAGTTG GATGAAAGCG AGAGTACTTG GCCTGCACGT AAACAAGTCG
 251 TTGCTTGTGC TGGTGTCTGC AAGACATGGA GACTAATGTG CAAAGATATT
 301 GTGAAAAGTC CTGAGTTCTC AGGCAAACTC ACATTTCCAG TTTCGTTGAA
 351 ACAGCCCGGG CCTAGGGATG GAATCATACA ATGTTATATC AAAAGAGACA
 401 AGTCTAACAT GACTTACCAC CTTTACCTTT CTCTTAGTCC TGCCATACTT
 451 GTTGAAAGTG GGAAGTTTCT TCTCTCGGCA AAGCGCTCAC GGAGAGCTAC
 501 ATACACAGAG TATGTAATAT CAATGGATGC AGACAACATT TCAAGATCAA
 551 GCAGCACTTA CATTGGCAAA CTGAAGTCTA ACTTTCTAGG GACAAAATTT
 601 ATAGTATATG ATACGGCTCC TGCGTACAAC AGTAGCCAGA TATTGTCCCC
 651 ACCAAACCGG AGTCGTAGTT TCAACTCCAA GAAAGTGTCT CCCAAAGTCC
 701 CTTCTGGAAG TTACAACATT GCTCAAGTTA CATACGAGCT GAACTTGCTT
 751 GGAACCCGTG GACCTCGTAG AATGAACTGC ATTATGCACT CTATCCCCTC
 801 CTTAGCTCTA GAACCCGGAG GTACTGTCCC TAGCCAACCT GAGTTTCTAC
 851 AACGTTCCCT TGATGAATCT TTCCGCAGCA TCGGTTCCTC AAAGATAGTC
 901 AACCACTCGG GAGATTTCAC CCGACCGAAA GAGGAAGAAG GAAAGGTGCG
 951 ACCTTTGGTA CTGAAAACTA AACCGCCAAG GTGGCTCCAA CCGTTGCGAT
1001 GTTGGTGCCT TAACTTCAAA GGGAGAGTGA CTGTAGCTTC TGTCAAGAAC
1051 TTCCAGTTGA TGTCCGCTGC AACGGTTCAG CCCGGTAGTG GTAGTGATGG
1101 TGGAGCATTG GCTACGAGGC CATCGTTATC ACCACAGCAG CCAGAGCAAT
1151 CAAACCATGA TAAGATAATA CTACACTTTG GGAAAGTGGG TAAGGATATG
1201 TTCACTATGG ACTATCGTTA TCCTCTCTCT GCCTTTCAAG CGTTTGCCAT
1251 TTCCCTGAGC ACCTTTGATA CTAAATTGGC ATGTGAA (SEQ ID NO: 16)
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AtTLP6:

polypeptide:

```
   1 MSLKNIVKNK YKAIGRRGRS HIAPEGSSVS SSLSTNEGLN QSIWVDLPPE
  51 LLLDIIQRIE SEQSLWPGRR DVVACASVCK SWREMTKEVV KVPELSGLIT
 101 FPISLRQPGP RDAPIQCFIK RERATGIYRL YLGLSPALSG DKSKLLLSAK
 151 RVRRATGAEF VVSLSGNDFS RSSSNYIGKL RSNFLGTKFT VYENQPPPFN
 201 RKLPPSMQVS PWVSSSSSSY NIASILYELN VLRTRGPRRM QCIMHSIPIS
 251 AIQEGGKIQS PTEFTNQGKK KKKPLMDFCS GNLGGESVIK EPLILKNKSP
 301 RWHEQLQCWC LNFKGRVTVA SVKNFQLVAA AAEAGKNMNI PEEEQDRVIL
 351 QFGKIGKDIF TMDYRYPISA FQAFAICLSS FDTKPVCE (SEQ ID NO:6)
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cDNA:

```
   1 ATGTCATTGA AGAACATAGT GAAGAACAAA TACAAAGCTA TTGGTAGAAG
  51 AGGGAGGTCA CACATTGCAC CAGAAGGATC ATCTGTGTCT TCTTCTTTAT
 101 CAACTAATGA AGGTTTAAAC CAGAGTATTT GGGTTGATTT GCCTCCAGAG
 151 TTACTTCTTG ATATAATCCA AAGGATTGAG TCTGAACAGA GTTTATGGCC
 201 GGGGAGGAGA GATGTTGTTG CTTGTGCTTC GGTTTGTAAG TCATGGAGGG
 251 AGATGACTAA AGAAGTTGTT AAAGTTCCTG AGCTCTCTGG TTTGATCACG
 301 TTTCCGATTT CTTTAAGACA GCCTGGACCT AGAGATGCTC CAATTCAATG
 351 CTTTATTAAA CGTGAAAGAG CTACGGGGAT ATACCGTCTC TATCTTGGTT
 401 TAAGCCCTGC TCTTTCCGGT GACAAGAGTA AGTTGTTGTT ATCTGCAAAG
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 451  AGAGTCAGGA GAGCGACGGG TGCGGAGTTT GTTGTATCGT TATCGGGGAA
 501  TGACTTCTCG AGAAGTAGTA GTAATTACAT AGGAAAACTG AGATCAAATT
 551  TCCTGGGAAC GAAGTTCACA GTCTACGAAA ACCAACCTCC TCCGTTTAAC
 601  CGAAAGCTCC CACCATCGAT GCAAGTGTCT CCATGGGTAT CGTCGTCATC
 651  TAGTAGTTAC AACATAGCTT CAATCTTGTA TGAGCTGAAT GTTCTGAGAA
 701  CCAGAGGTCC AAGAAGAATG CAATGTATAA TGCACAGTAT CCCGATTTCA
 751  GCGATTCAAG AAGGCGGCAA AATCCAGTCG CCAACGGAGT TCACAAACCA
 801  AGGAAAGAAG AAGAAGAAGC CGCTGATGGA TTTCTGCTCA GGGAACCTGG
 851  GAGGAGAATC CGTTATAAAA GAACCATTAA TTCTGAAAAA CAAGTCGCCG
 901  AGATGGCACG AACAGCTTCA GTGCTGGTGT CTAAACTTCA AAGGTCGAGT
 951  CACAGTCGCC TCGGTGAAAA ACTTCCAGCT AGTGGCAGCT GCTGCAGAAG
1001  CAGGGAAGAA CATGAACATA CCAGAAGAGG AACAAGATAG AGTGATATTA
1051  CAGTTTGGGA AGATAGGCAA AGACATTTTC ACAATGGATT ATCGTTACCC
1101  GATCTCTGCA TTCCAAGCTT TTGCTATTTG TTTAAGCAGC TTCGACACGA
1151  AGCCAGTCTG CGAA  (SEQ ID NO:17)
```

AtTLP7:

polypeptide:

```
   1  MPLSRSLLSR RISNSFRFHQ GETTTAPESE SIPPPSNMAG SSSWSAMLPE
  51  LLGEIIRRVE ETEDRWPQRR DVVTCACVSK KWREITHDFA RSSLNSGKIT
 101  FPSCLKLPGP RDFSNQCLIK RNKKTSTFYL YLALTPSFTD KGKFLLAARR
 151  FRTGAYTEYI ISLDADDFSQ GSNAYVGKLR SDFLGTNFTV YDSQPPHNGA
 201  KPSNGKASRR FASKQISPQV PAGNFEVGHV SYKFNLLKSR GPRRMVSTLR
 251  CPSPSPSSSS AGLSSDQKPC DVTKIMKKPN KDGSSLTILK NKAPRWHEHL
 301  QCWCLNFHGR VTVASVKNFQ LVATVDQSQP SGKGDEETVL LQFGKVGDDT
 351  FTMDYRQPLS AFQAFAICLT SFGTKLACE  (SEQ ID NO:7)
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cDNA:

```
   1  ATGCCTTTGT CACGGTCCCT CCTTTCGCGG AGGATCTCGA ACTCTTTTAG
  51  GTTTCATCAG GGAGAGACAA CGACGGCACC GGAATCCGAA TCGATTCCTC
 101  CGCCGTCGAA TATGGCCGGT TCTTCGTCAT GGTCGGCGAT GCTCCCTGAA
 151  TTATTAGGCG AGATCATTCG TCGCGTGGAG GAGACTGAGG ACCGTTGGCC
 201  TCAACGTCGT GATGTAGTTA CTTGCGCTTG CGTTTCTAAG AAATGGAGAG
 251  AAATCACTCA CGATTTCGCT AGATCCTCTC TTAACTCTGG CAAAATTACT
 301  TTCCCTTCTT GCCTCAAATT GCCAGGTCCT AGAGACTTTT CTAATCAGTG
 351  CTTGATAAAG AGGAACAAGA AGACATCAAC GTTTTACTTG TATCTTGCTC
 401  TAACACCATC ATTCACTGAT AAGGGAAAGT TTCTTCTGGC GGCGCGGAGG
 451  TTTAGGACCG GTGCTTACAC TGAGTACATC ATATCACTTG ATGCTGATGA
 501  TTTCTCTCAA GGAAGTAATG CCTACGTCGG AAAATTAAGA TCAGATTTTC
 551  TTGGGACCAA CTTTACAGTA TACGATAGCC AACCACCACA CAACGGAGCA
 601  AAACCTTCAA ATGGCAAAGC CAGTCGCAGA TTTGCATCAA AGCAGATAAG
 651  CCCTCAAGTT CCAGCAGGCA ACTTTGAAGT CGGTCATGTT TCTTATAAAT
 701  TCAACCTTTT GAAATCAAGA GGTCCAAGAA GAATGGTAAG CACACTCCGA
 751  TGCCCATCAC CATCACCTTC ATCATCATCC GCTGGACTCT CGTCTGACCA
 801  AAAGCCATGT GATGTAACCA AGATAATGAA AAAACCCAAC AAGGATGGTT
 851  CCAGCTTGAC AATACTAAAG AACAAAGCTC CTAGATGGCA CGAGCACTTG
 901  CAGTGCTGGT GTCTGAACTT CCATGGACGA GTTACTGTTG CTTCGGTCAA
 951  GAACTTTCAG CTGGTTGCGA CCGTTGACCA AAGTCAACCG AGCGGTAAAG
```

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1001  GCGATGAAGA AACAGTTCTT CTACAGTTTG GTAAAGTGGG AGATGACACT
1051  TTCACTATGG ATTATAGACA GCCTCTCTCT GCATTTCAGG CTTTTGCTAT
1101  CTGTCTCACA AGTTTCGGCA CTAAACTTGC CTGCGAG (SEQ ID NO:18)
```

AtTLP8:

polypeptide:

```
   1  MAGSRKVNDL LEENKGNVDT ITGSLSTQKG EDKENVSPEK VSTSVETRKL
  51  DRALKSQSMK GNSGFPTEVT NFKSFSTGGR TALKQSSLQA CMQKNSEVDK
 101  SSFGMKTWTS VDSEHSSSLK VWEFSDSEAA PASSWSTLPN RALLCKTLPL
 151  DVGRCTCLIV KEQSPEGLSH GSVYSLYTHE GRGRKDRKLA VAYHSRRNGK
 201  SIFRVAQNVK GLLCSSDESY VGSMTANLLG SKYYIWDKGV RVGSVGKMVK
 251  PLLSVVIFTP TITTWTGSYR RMRTLLPKQQ PMQKNNNKQV QQASKLPLDW
 301  LENKEKIQKL CSRIPHYNKI SKQHELDFRD RGRTGLRIQS SVKNFQLTLT
 351  ETPRQTILQM GRVDKARYVI DFRYPFSGYQ AFCICLASID SKLCCTV  (SEQ ID NO:8)
```

cDNA:

```
   1  ATGGCTGGTT CGAGAAAAGT GAATGATTTG TTGGAGGAAA ATAAGGGAAA
  51  TGTGGACACA ATTACAGGGT CTTTATCCAC TCAAAAGGGA GAGGATAAGG
 101  AGAATGTGTC GCCGGAGAAA GTCTCTACCT CTGTGGAAAC TCGGAAACTA
 151  GATCGAGCTT TGAAGTCTCA ATCGATGAAG GGTAACTCTG GGTTTCCAAC
 201  GGAAGTTACA AATTTCAAAT CTTTCTCAAC TGGTGGTCGA ACAGCTCTGA
 251  AGCAGTCATC ACTGCAAGCG TGTATGCAGA AGAACAGTGA GGTTGATAAG
 301  AGTAGTTTCG GAATGAAAAC TTGGACTAGT GTTGATTCAG AGCATTCAAG
 351  TTCGTTGAAA GTGTGGGAGT TTTCGGATTC TGAAGCTGCC CCTGCTTCCT
 401  CTTGGTCTAC TTTGCCCAAC AGGGCTTTGT TGTGCAAGAC ACTACCTTTG
 451  GATGTGGGAA GATGCACTTG TCTGATTGTG AAAGAACAAT CACCTGAAGG
 501  CTTGAGCCAC GGATCTGTAT ATTCACTTTA TACACATGAA GGTCGGGGGC
 551  GTAAAGACCG GAAGTTAGCA GTTGCTTACC ATAGCCGACG TAATGGGAAA
 601  TCTATATTTA GGGTGGCACA GAATGTTAAG GGATTGCTGT GCAGTTCGGA
 651  TGAAAGTTAT GTCGGTTCCA TGACGGCTAA TCTCTTGGGT TCCAAGTACT
 701  ACATATGGGA CAAGGGAGTT CGAGTTGGTT CTGTAGGTAA AATGGTGAAG
 751  CCGCTTCTTT CGGTTGTAAT ATTCACACCC ACCATAACAA CTTGGACAGG
 801  GAGCTACAGA AGAATGAGAA CTTTGCTACC AAAGCAGCAG CCAATGCAGA
 851  AAAACAACAA TAAGCAGGTT CAACAAGCTA GTAAACTACC GCTTGATTGG
 901  CTTGAGAATA AGGAAAAAAT TCAGAAGCTA TGCTCAAGGA TACCACATTA
 951  CAACAAAATC TCCAAGCAGC ATGAGTTAGA CTTCAGAGAC AGAGGAAGAA
1001  CAGGACTGAG AATACAGAGC TCGGTGAAGA ACTTTCAGCT AACACTCACG
1051  GAGACTCCAA GGCAGACAAT TCTTCAAATG GGGAGAGTTG ACAAAGCAAG
1101  ATATGTAATC GACTTCAGGT ATCCATTCTC AGGCTACCAA GCATTCTGCA
1151  TTTGCTTGGC TTCTATTGAT TCCAAGCTTT GTTGTACTGT T  (SEQ ID NO:19)
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AtTLP9:

polypeptide:

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   1  MTFRSLLQEM RSRPHRVVHA AASTANSSDP FSWSELPEEL LREILIRVET
  51  VDGGDWPSRR NVVACAGVCR SWRILTKEIV AVPEFSSKLT FPISLKQSGP
 101  RDSLVQCFIK RNRNTQSYHL YLGLTTSLTD NGKFLLAASK LKRATCTDYI
 151  ISLRSDDISK RSNAYLGRMR SNFLGTKFTV FDGSQTGAAK MQKSRSSNFI
 201  KVSPRVPQGS YPIAHISYEL NVLGSRGPRR MRCIMDTIPM SIVESRGVVA
 251  STSISSFSSR SSPVFRSHSK PLRSNSASCS DSGNNLGDPP LVLSNKAPRW
 301  HEQLRCWCLN FHGRVTVASV KNFQLVAVSD CEAGQTSERI ILQFGKVGKD
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351 MFTMDYGYPI SAFQAFAICL SSFETRIACE (SEQ ID NO:9)

cDNA:

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   1 ATGACGTTCC GAAGTTTACT CCAGGAAATG CGGTCTAGGC CACACCGTGT
  51 AGTTCACGCC GCCGCCTCAA CCGCTAATAG TTCAGACCCT TTCAGCTGGT
 101 CGGAGCTCCC GGAGGAGCTG CTTAGAGAAA TCCTGATTAG GGTTGAGACT
 151 GTTGACGGCG GCGATTGGCC GTCGCGGCGA AACGTGGTGG CTTGTGCCGG
 201 CGTTTGTCGT AGCTGGAGGA TTCTCACCAA GGAGATTGTA GCTGTTCCTG
 251 AATTCTCCTC TAAATTGACT TTCCCTATCT CCCTCAAGCA GTCTGGTCCA
 301 AGAGATTCTC TAGTTCAATG CTTTATAAAA CGTAATCGAA ATACTCAATC
 351 GTATCATCTC TATCTCGGAT TAACTACCTC TTTGACGGAT AACGGGAAGT
 401 TTCTTCTTGC TGCTTCTAAG CTGAAGCGCG CAACTTGCAC TGATTACATC
 451 ATCTCTTTGC GTTCAGACGA TATCTCAAAG AGAAGCAACG CGTATCTTGG
 501 GAGAATGAGA TCGAACTTCC TTGGAACAAA ATTCACGGTC TTTGATGGTA
 551 GTCAGACCGG AGCAGCGAAG ATGCAGAAGA GCCGCTCTTC TAATTTCATC
 601 AAAGTTTCAC CTAGAGTTCC TCAGGGAAGT TACCCCATCG CTCACATTTC
 651 ATACGAGTTA AACGTCTTAG GCTCTCGGGG ACCGAGAAGA ATGCGTTGCA
 701 TCATGGATAC AATACCTATG AGCATCGTGG AGTCGCGAGG AGTAGTAGCT
 751 TCAACATCCA TAAGCTCTTT TTCCAGTCGG TCATCACCAG TCTTTAGGTC
 801 TCACTCAAAA CCATTGCGCA GTAATAGTGC ATCATGTAGC GACTCAGGCA
 851 ACAACCTGGG AGATCCACCA TTGGTGCTGA GCAACAAAGC TCCACGGTGG
 901 CATGAGCAGT TACGTTGCTG GTGCTTAAAT TTCCATGGTC GAGTCACAGT
 951 GGCTTCGGTT AAGAACTTTC AGCTTGTGGC AGTTAGTGAC TGTGAAGCAG
1001 GGCAGACATC TGAGAGGATC ATACTCCAGT TTGGGAAAGT TGGGAAGGAC
1051 ATGTTTACCA TGGATTATGG ATATCCGATT TCTGCGTTTC AAGCGTTTGC
1101 TATCTGCCTG AGCAGTTTTG AAACCAGAAT TGCCTGTGAA (SEQ ID NO:20)
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AtTLP10:

polypeptide:

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   1 MSFRGIVQDL RDGFGSLSRR SFDFRLSSLH KGKAQGSSFR EYSSSRDLLS
  51 PVIVQTSRWA NLPPELLFDV IKRLEESESN WPARKHVVAC ASVCRSWRAM
 101 CQEIVLGPEI CGKLTFPVSL KQPGPRDAMI QCFIKRDKSK LTFHLFLCLS
 151 PALLVENGKF LLSAKRTRRT TRTEYIISMD ADNISRSSNS YLGKLRSNFL
 201 GTKFLVYDTQ PPPNTSSSAL ITDRTSRSRF HSRRVSPKVP SGSYNIAQIT
 251 YELNVLGTRG PRRMHCIMNS IPISSLEPGG SVPNQPEKLV PAPYSLDDSF
 301 RSNISFSKSS FDHRSLDFSS SRFSEMGISC DDNEEEASFR PLILKNKQPR
 351 WHEQLQCWCL NFRGRVTVAS VKNFQLVAAR QPQPQGTGAA AAPTSAPAHP
 401 EQDKVILQFG KVGKDMFTMD YRYPLSAFQA FAICLSSFDT KLACE (SEQ ID NO:10)
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cDNA:

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   1 ATGTCGTTTC GAGGCATTGT TCAAGATTTG AGAGATGGGT TTGGGAGCTT
  51 GTCAAGGAGG AGTTTCGATT TTAGGCTCTC GAGTCTTCAT AAAGGGAAAG
 101 CTCAGGGTTC TTCGTTCCGT GAGTATTCGT CATCCCGTGA TCTCTTGTCG
 151 CCTGTGATAG TTCAGACAAG TAGATGGGCT AATCTTCCTC CAGAGTTACT
 201 CTTTGATGTG ATCAAAAGAT TAGAGGAAAG TGAGAGTAAT TGGCCTGCAA
 251 GAAAACATGT TGTGGCTTGT GCTTCGGTTT GTCGGTCTTG GAGAGCTATG
 301 TGCCAAGAGA TTGTTTTGGG GCCTGAAATC TGTGGGAAAC TCACTTTCCC
 351 TGTTTCCCTC AAACAGCCAG GGCCTCGTGA TGCAATGATT CAGTGTTTCA
 401 TCAAAAGGGA TAAATCAAAG CTAACATTTC ACCTTTTTCT TTGTTTAAGT
 451 CCCGCTCTAT TAGTGGAGAA TGGGAAATTT CTTCTTTCAG CTAAAAGAAC
 501 TCGTAGAACT ACTCGAACCG AGTACATTAT CTCCATGGAT GCTGATAACA
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 551  TCTCAAGATC CAGCAACTCT TACCTCGGAA AGCTCAGATC AAACTTCCTT
 601  GGGACAAAGT TCTTGGTGTA CGACACGCAA CCACCACCAA ACACATCTTC
 651  GAGCGCACTT ATCACTGATC GAACAAGCCG AAGCAGGTTT CACTCCAGAC
 701  GAGTTTCTCC TAAAGTACCA TCCGGAAGCT ACAACATTGC TCAAATCACC
 751  TATGAGCTCA ACGTGTTGGG CACACGCGGG CCACGACGAA TGCACTGCAT
 801  CATGAACTCC ATCCCAATTT CATCGCTCGA ACCAGGCGGT TCAGTCCCTA
 851  ACCAACCCGA GAAACTCGTC CCTGCACCAT ACTCTCTCGA CGACTCATTC
 901  CGCAGTAACA TCTCCTTCTC CAAATCATCA TTTGACCACC GCTCCCTCGA
 951  TTTCAGCAGT TCTAGATTCT CCGAAATGGG AATATCCTGC GACGACAACG
1001  AAGAAGAAGC GAGTTTCAGA CCGTTGATTC TAAAGAACAA GCAGCCAAGG
1051  TGGCACGAGC AGTTGCAATG CTGGTGTTTG AATTTCCGCG GACGTGTGAC
1101  AGTTGCATCG GTTAAGAATT TCCAGCTTGT AGCAGCAAGA CAGCCGCAGC
1151  CTCAAGGGAC AGGTGCAGCA GCAGCACCAA CAAGTGCACC TGCTCACCCT
1201  GAGCAAGACA AGGTGATTCT CCAGTTTGGT AAAGTAGGGA AAGATATGTT
1251  CACAATGGAC TATAGGTATC CATTATCGGC GTTTCAGGCG TTTGCGATAT
1301  GCTTAAGCAG CTTTGACACC AAGCTTGCTT GTGAA  (SEQ ID NO:21)
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AtTLP11:

polypeptide:

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   1  MRSRPHRVVH DLAAAAAADS TSVSSQDYRW SEIPEELLRE ILIRVEAADG
  51  GGWPSRRSVV ACAGVCRGWR LLMNETVVVP EISSKLTFPI SLKQPGPRDS
 101  LVQCFIKRNR ITQSYHLYLG LTNSLTDDGK FLLAACKLKH TTCTDYIISL
 151  RSDDMSRRSQ AYVGKVRSNF LGTKFTVFDG NLLPSTGAAK LRKSRSYNPA
 201  KVSAKVPLGS YPVAHITYEL NVLGSRGPRK MQCLMDTIPT STMEPQGVAS
 251  EPSEFPLLGT RSTLSRSQSK PLRSSSSHLK ETPLVLSNKT PRWHEQLRCW
 301  CLNFHGRVTV ASVKNFQLVA AGASCGSGTG MSPERQSERI ILQFGKVGKD
 351  MFTMDYGYPI SAFQAFAICL SSFETRIACE  (SEQ ID NO:11)
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cDNA:

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   1  ATGCGTTCGA GACCGCATCG TGTGGTCCAC GACCTTGCCG CCGCCGCAGC
  51  TGCCGATTCC ACTTCTGTGT CATCGCAAGA TTATCGCTGG TCAGAGATTC
 101  CTGAAGAGCT TCTTAGGGAG ATTCTGATTC GTGTTGAAGC GGCGGACGGT
 151  GGCGGATGGC CGTCACGACG CAGCGTGGTG GCTTGTGCCG GCGTTTGTCG
 201  TGGCTGGCGG CTACTTATGA ACGAAACCGT CGTTGTCCCT GAGATCTCTT
 251  CTAAGTTGAC TTTCCCCATC TCTCTCAAGC AGCCTGGTCC AAGGGATTCA
 301  CTGGTTCAAT GCTTTATCAA ACGTAATCGA ATTACGCAAT CATATCATCT
 351  CTATCTCGGA TTAACCAACT CTTTAACGGA TGATGGGAAG TTTTTGCTTG
 401  CTGCGTGTAA GTTGAAGCAC ACAACTTGTA CGGATTACAT TATCTCTTTA
 451  CGTTCTGATG ATATGTCGAG AAGAAGCCAA GCTTATGTTG GCAAAGTGAG
 501  ATCGAACTTC CTAGGAACGA AATTCACTGT CTTTGATGGA AATCTGCTGC
 551  CTTCAACGGG AGCCGCAAAG TTGAGAAAGA GCCGATCTTA TAATCCCGCA
 601  AAAGTTTCAG CAAAAGTTCC TCTTGGAAGT TATCCTGTCG CTCATATCAC
 651  ATATGAGCTG AATGTCTTAG GATCCCGGGG ACCAAGAAAG ATGCAATGTC
 701  TTATGGACAC AATACCTACA AGCACAATGG AGCCTCAAGG AGTAGCTTCA
 751  GAACCATCAG AGTTTCCCTT ACTCGGTACT CGGTCAACCT TATCCAGGTC
 801  TCAGTCAAAA CCATTACGCA GTAGCTCAAG CCACCTGAAA GAAACACCAT
 851  TAGTGCTGAG CAACAAGACA CCACGGTGGC ACGAGCAGCT ACGCTGCTGG
 901  TGCTTGAATT TCCATGGCCG TGTCACAGTA GCGTCAGTGA AGAACTTTCA
 951  GCTCGTGGCA GCAGGAGCTA GCTGTGGCAG TGGCACGGGA ATGTCACCGG
1001  AGAGGCAGAG CGAGCGGATT ATATTGCAGT TTGGGAAAGT CGGGAAAGAT
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1051 ATGTTCACGA TGGATTATGG ATACCCGATC TCAGCTTTCC AGGCTTTTGC
1101 CATTTGCTTG AGCAGCTTTG AGACTAGAAT CGCTTGTGAA (SEQ ID NO:22)

Accordingly, one aspect of the invention features an isolated polypeptide containing an amino acid sequence at least 70% (i.e., any number between 70% and 100%, inclusive) identical to one of SEQ ID NOs: 1-11. When expressed in a plant cell, e.g., an *Arabidopsis* cell, the polypeptide regulates the transcription of genes, in response to environmental stimuli. The polypeptide of the invention can be used to identify DNA elements, such as promoters, enhances, or silencers, which it binds to. Such DNA elements mediate the response of plants to various environmental factors. The polypeptide of the invention can also be used for producing anti-AtTLP antibodies (either monoclonal or polyclonal). These antibodies in turn are useful for detecting the presence and distribution of AtTLP proteins in tissues and in cellular compartments. For example, such antibodies can be used to verify the expression of TLP proteins in a transgenic plant.

An isolated polypeptide refers to a polypeptide substantially free from naturally associated molecules, i.e., it is at least 75% (i.e., any number between 75% and 100%, inclusive) pure by dry weight. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The percent identity of two amino acid sequences is determined using the algorithm of Karlin and Altschul ((1990) Proc. Natl. Acad. Sci. USA 87, 2264-2268), modified as in Karlin and Altschul ((1993) Proc. Natl. Acad. Sci. USA 90, 5873-5877). Such an algorithm is incorporated into the XBLAST programs of Altschul, et al. ((1990) J. Mol. Biol. 215, 403-410). BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3. Where gaps exist between two sequences, Gapped BLAST is utilized as described in Altschul, et al. ((1997) Nucleic Acids Res. 25, 3389-3402). When employing BLAST and Gapped BLAST programs, one can conveniently use the default parameters (e.g., XBLAST). See ncbi.nlm.nih.gov.

The invention further features (1) an isolated nucleic acid encoding a polypeptide of the invention and (2) an isolated nucleic acid that, under a high stringent condition, hybridizes to a probe containing a sequence selected from the group consisting of SEQ ID NOs: 12-22, or a complement of any selected sequence. Such a nucleic acid is at least 15 (e.g., at least 30, 50, 100, 200, 500, or 1000) nucleotides in length. By hybridization under a high stringent condition

is meant hybridization at 65°C, 0.5 X SSC, followed by washing at 45°C, 0.1 X SSC. The nucleic acids of the invention can be used to determine whether an AtTLP mRNA is expressed in a tissue or cell. The nucleic acids can be used as primers in PCR-based detection methods, or as labeled probes in nucleic acid blots (e.g., Northern blots).

An isolated nucleic acid refers to a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid. The term therefore covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein.

The invention also features a vector and a host cell containing a nucleic acid of the invention. The host cell can be an E. coli., a yeast, an insect, a plant (e.g., *Arabidopsis*), or a mammalian cell. The vector and host cell can be used for producing a polypeptide of the invention. For this purpose, one can culture the host cell in a medium under conditions permitting expression of the polypeptide, and isolate the polypeptide.

The just-described vector and host cell can also be used for generating a transformed plant cell or a transgenic plant containing a recombinant nucleic acid that encodes a heterologous polypeptide of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. One can generate such a transformed plant cell by introducing into a plant cell a recombinant nucleic acid encoding such a heterologous polypeptide and expressing the polypeptide in the cell. To generate a transgenic plant, one can (1) introduce into a plant cell a recombinant nucleic acid encoding one just-described heterologous polypeptide; (2) expressing the polypeptide in the cell, and (3) cultivating the cell to generate a plant. The transformed plant cell or transgenic plant is more sensitive to environmental factors, such as high salinity, pathogens, and chilling, and therefore can be used as a sensor to detect and monitor small changes in environment, such as soil and air.

Also within the scope of this invention are a homozygous transformed plant cell (e.g., an

Arabidopsis cell) and a transgenic plant (e. g. Arabidopsis) that lack a polypeptide containing a sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. The transformed plant cell or transgenic plant, compared with the wild type cell or plant, has a higher (by at least 30%, e.g., 50%, 90%, 100%, 200%) tolerance to salt, chilling, pathogens, oxidative stress, or water-deficit due to absence of or lowered level of the polypeptide. In addition, the invention features method of making the transformed plant cell and the transgenic plant. Both methods include introducing into a plant cell a nucleic acid (e.g., a T-DNA, an anti-sense RNA, and an iRNA) that decreases the expression of a gene encoding a polypeptide of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. The method for making the plant further includes cultivating the plant cell to generate a plant.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DETAILED DESCRIPTION

This invention is based on an unexpected discovery that (1) overexpression of AtTLPs in *Arabidopsis* increases sensitivity of the plant to various environmental factors, such as salt, chilling, oxidative stress, or water-deficit; and (2) lack of expression of AtTLPs increases tolerance of the plant to several environmental factors.

Accordingly, in one aspect, the invention features a transformed plant cell containing a recombinant nucleic acid that encodes a heterologous AtTLP. The AtTLP proteins useful for this invention include, *Arabidopsis* AtTLPs 1-11 and TLPs of other species. The plant cell can be a dicot plant cell (e.g., a tomato cell, a brassica cell, or a potato cell) or a monocot plant cell (e.g. a rice cell, a wheat cell, or a barley cell).

A transformed plant cell of the invention can be produced by introducing into a plant cell a recombinant nucleic acid that encodes a heterologous AtTLP protein and expressing the protein in the cell. Techniques for transforming a wide variety of plant cells are well known in the art and can be found in technical and scientific literature. See, for example, Weising et al., 1988, Ann. Rev. Genet. 22:421-477. To express a heterologous AtTLP gene in a plant cell, the gene can be combined with transcriptional and translational initiation regulatory sequences that direct the transcription of the gene and translation of the encoded protein in the plant cell.

For overexpression, a constitutive plant promoter may be employed. A constitutive

promoter is active under most environmental conditions and states of cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'-promoter derived from T-DNA of Agrobacterium tumafaciens, the ACT11 and Cat3 promoters from Arabidopsis (Huang et al., 1996, Plant Mol. Biol. 33:125-139 and Zhong et al., 1996, Mol. Gen. Genet. 251:196-203), the stearoyl-acyl carrier protein desaturase gene promoter from Brassica napus (Solocombe et al., 1994, Plant Physiol. 104:1167-1176), and the GPc1 and Gpc2 promoters from maize (Martinez et al., 1989, J. Mol. Biol. 208:551-565 and Manjunath et al., 1997, Plant Mol. Biol. 33:97-112).

Alternatively, a tissue-specific promoter or an inducible promoter may be employed to direct expression of the AtTLP gene in a specific cell type or under more precise environmental or developmental control. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobicity, elevation of temperature, presence of light, spray with chemicals or hormones, or infection by a pathogen. Examples of a tissue-specific promoter or an inducible promoter include the root-specific ANR1 promoter (Zhang and Forde, 1998, Science 279:407) and the photosynthetic organ-specific RBCS promoter (Khoudi et al., 1997, Gene 197:343).

For proper polypeptide expression, a polyadenylation region at the 3'-end of the coding region should be included. The polyadenylation region can be derived from the same gene, from a variety of other genes, or from T-DNA.

A marker gene can also be included to confer a selectable phenotype on plant cells. For example, the marker gene may encode a protein that confers biocide resistance, antibiotic resistance (e.g., resistance to kanamycin, G418, bleomycin, hygromycin), or herbicide resistance (e.g., resistance to chlorosulfuron or Basta).

A recombinant nucleic acid that encodes a heterologous AtTLP protein may be introduced into the genome of a desired plant host cell by a variety of conventional techniques. For example, the recombinant nucleic acid may be introduced directly into the genomic DNA of a plant cell using techniques such as polyethylene glycol precipitation, electroporation, microinjection, or ballistic methods (e.g., DNA particle bombardment). See, e.g., Paszkowski et al., 1984, EMBO J. 3:2717-2722, Fromm et al., 1985, Proc. Natl. Acad. Sci. USA 82:5824, and Klein et al., 1987, Nature 327:70-73. Alternatively, the recombinant nucleic acid may be combined with suitable T-DNA flanking regions and introduced into a conventional

Agrobacterium tumefaciens host vector. The virulence functions of the Agrobacterium tumefaciens host direct the insertion of the AtTLP gene and adjacent marker into the plant cell DNA when the cell is infected by the bacteria. Agrobacterium tumefaciens-mediated transformation techniques, including disarming and use of binary vectors, are well known in the art. See, e.g., Horsch et al., 1984, Science 233:496-498; Fraley et al., 1983, Proc. Natl. Acad. Sci. USA 80:4803; and Gene Transfer to Plants, Potrykus, ed., Springer-Verlag, Berlin, 1995.

The presence and copy number of a heterologous AtTLP gene in a transgenic plant can be determined using standard methods, e.g., Southern blotting. Expression of the heterologous AtTLP gene in a transgenic plant can be confirmed by detecting and quantifying the heterologous AtTLP mRNA or protein in the transgenic plant.

The transformed plant cells thus obtained can then be cultured to regenerate a whole plant. Regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide or herbicide marker that has been introduced together with a heat shock factor gene. Plant regeneration from cultured protoplasts is described in Evans et al., Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, pp. 124-176, MacMillilan Publishing Company, New York, 1983; and Binding, Regeneration of Plants, Plant Protoplasts, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al., 1987, Ann. Rev. Plant Phys. 38:467-486. Once the heterologous AtTLP gene has been confirmed to be stably incorporated in the genome of a transgenic plant, it can be introduced into other plants by sexual crossing. Depending upon the species to be crossed, one or more standard breeding techniques can be used to generate the whole plant.

In another aspect, the invention feature a homozygous transformed plant cell that lack one or more of AtTLPs 1-11. Absence of the AtTLP(s) enhances tolerance of the cell to various environmental factors, e.g., high salinity. Such a transformed cell can be made by introducing into a plant cell a nucleic acid that lowers the expression of a gene encoding a polypeptide of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. The nucleic acid, e.g., T-DNA, antisense RNA, or iRNA, can be introduced into the cell using one of the standard transforming techniques described above. Stable transformants can be selected using the marker genes and selection methods also described above. A whole plant can then be regenerated from the transformed plant cells. It can be further crossed using conventional breeding techniques to generate

homozygous plant.

The specific example below is to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. All publications cited herein are hereby incorporated by reference in their entirety.

Identification of the AtTLP Family

A tubby consensus sequence (Pfam PF01167, Kleyn et al., 1996, Cell 85: 281-290 and Noben-Trauth et al., 1996, Nature 380: 534-538) was used to search the *Arabidopsis thaliana* expressed sequence tag (EST) database and the completed Arabidopsis genome sequence (The Institute of Genome Research, TIGR) with multiple BLAST algorithms to locate all the sequences sharing significant similarities with the tubby domain (P-value < 0.0085). The search results revealed eleven TUBBY-like protein genes, termed AtTLP1 to AtTLP11, in the Arabidopsis genome. For each of the 11 genes, the corresponding BAC locus (The Arabidopsis Information Resource), Tentative Consensus (TC) group, AGI gene code, cDNA GenBank accession number, and predicted protein length (No. of amino acid) are summarized in Table 1 below.

Table 1. AtTLP family members

Gene Name	BAC Locus	TC group	AGI Gene Code	cDNA GenBank Accession No.	Predicted Protein Length
AtTLP1	F22K20.1	TC95487	At1g76900	AF487267	455
AtTLP2	T30D6.21	TC86308	At2g18280	AY045773	394
AtTLP3	F17A22.29	TC86633	At2g47900	AY045774	406
AtTLP4	F8K4.13	-	At1g61940	-	265
AtTLP5	T10P12.9	TC102456	At1g43640	AY046921	429
AtTLP6	F8G22.1	TC90700	At1g47270	AF487268	388
AtTLP7	F12M16.22	TC88599	At1g53320	AY092403	379
AtTLP8	T24D18.17	-	At1g16070	AF487269	397
AtTLP9	F24P17.15	TC102624	At3g06380	AF487270	380
AtTLP10	F4F7.13.	TC101291	At1g25280	AF487271	445
AtTLP11	T1A4.60	-	At5g18680	AY046922	380

Gene-specific 5' and 3' primers were designed based on the sequence of the predicted open reading frame (ORF) and the corresponding EST in the database. The primer pairs used are listed below:

AtTLP1-5' (5'-ATGTCGTTCCGTAGCATAGTTCGT-3')
AtTLP1-3' (5'-TTATTCGCAAGCAAGTTTTGTGTCG-3')
AtTLP2-5' (5'-ATGTCTTTGAAAAGCATCCTTCGTGATC-3')
AtTLP2-3' (5'-TTACCCTTCACATGCCGGTTTGGTGTCA-3')
AtTLP3-5' (5'-ATGTCCTTCAAGAGTCTCATTCAG-3')
AtTLP3-3' (5'-TCATTCACATGCTATCTTGGTGTC-3')
AtTLP5-5' (5'-ATGTCGTTTCTGAGTATTGTTCG-3')
AtTLP5-3' (5'-TTATTCACATGCCAATTTAGTAT-3')
AtTLP6-5' (5'-ATGTCATTGAAGAACATAGTGAA-3')
AtTLP6-3' (5'-TCATTCGCAGACTGGCTTCGTGT-3')
AtTLP7-5' (5'-ATGCCTTTGTCACGGTCCCTC-3')
AtTLP7-3' (5'-TCACTCGCAGGCAAGTTTAGTG-3')
AtTLP8-5' (5'-ATGGCTGGTTCGAGAAAAGTGAA-3')
AtTLP8-3' (5'-TCAAACAGTACAACAAAGCTTGG-3')
AtTLP9-5' (5'–ATGACGTTCCGAAGTTTACTCCA-3')
AtTLP9-3' (5'-TTATTCACAGGCAATTCTGGTTT-3')
AtTLP10-5' (5'-ATGTCGTTTCGAGGCATTGTTCA-3')
AtTLP10-3' (5'-CTATTCACAAGCAAGCTTGGTGT-3')
AtTLP11-5' (5'-ATGTCGTTTCTGAGTATTGTTCG-3')
AtTLP11-3' (5'-TTATTCACATGCCAATTTAGTAT-3')

RT-PCR was then performed using total RNA from-2-week-old Arabidopsis seedlings. The total RNA was isolated using the TRIZOL reagent (Invitrogen) according the manufacture's direction. PolyA$^+$-mRNA was isolated using oligo (dT)-coated magnetic beads and the PolyATract system (Promega, Madison, WI). First strand cDNA was synthesized from 0.5 μg PolyA$^+$-mRNA using SuperScript II RNase H Reverse Transcriptase (Invitrogen) according to the protocol of the supplier.

The above-described gene-specific primer pairs were used for amplifying cDNA of each AtTLP gene from first-strand cDNA. PCR conditions were as follows: 3 min at 94°C; 25 cycles of 1 min denaturation at 94°C/ 1 min annealing at 55°C/1 min 30 s extension at 72°C. The PCR products were purified using the QIAquick PCR purification kit (Qiagen) and subcloned into a T-easy vector (Promega). Each of these clones was verified by sequencing. Ten AtTLP cDNAs, AtTLPs 1-3 and AtTLPs 5-11, were successfully amplified.

It was found that, except for AtTLPs 2and 11, the amino acid sequences deduced from the cDNA sequences of AtTLPs 1, 3, and 5-10 are identical to the predicted ORFs in the database. The analysis of the AtTLP2 cDNA sequence indicated that its intron3 was located between 708-781 bp whereas the predicted splicing sites for this intron located were 663 and 766 bp. The analysis of AtTLP11 cDNA sequence showed that intron2 and intron4 were located at 669-803 bp and 1334-1575 bp, respectively, whereas the computer predicted intron2 was at 621-803 bp and there was no predicted intron4. All cDNA sequences obtained from this study were submitted to GenBank.

Sequence Analysis of AtTLP Proteins

The search for all known motifs in the deduced AtTLP amino acid sequences was conducted by MOTIF SCANNING (Pagni et al., 2001, Nucleic Acids Res 29: 148-151). Multiple sequence alignment was performed using ClustalW (Thomopson et al., 1994). This analysis reveled that each AtTLP gene, except AtTLPs 4 and 8, had a well-conserved tubby domain at its C-terminus. Unlike animal TLPs, which have highly diverse N-terminal sequences, each AtTLP, except AtTLP8, had a conserved F-box (51-57 residues) containing domain (Pfam PF00646).

Pair-wise comparisons among the AtTLP proteins revealed that their tubby domains shared 30% to 80% similarities. Further analyzing the tubby domain revealed two PROSITE signature patterns: TUB1 (Prosite Accession No. PS01200) and TUB2 motif (Prosite Accession No. PS01201). The TUB1 and TUB2 motifs were located at the C-terminal of each AtTLP protein and contain 14 and 16 amino acid residues, respectively. These two TUB motifs are highly conserved among TLPs from various organisms. Though AtTLP4 and 8 do not have obvious TUB1 and TUB2 motifs, their C-terminal tubby domains are recognizable by MOTIF SCANNING (N-score >15) (Pagni et al., 1993, Nucleic Acids Res 29: 148-151).

An obvious feature of AtTLPs is the tubby domain. In the tubby domain of a mouse TUBBY protein, three positively-charged amino acid residues, R332, R363 and K330, were thought to be crucial for PI (4,5) P_2 binding (Santagata et al., 2001, Science 292: 2041-2050). A sequence alignment of AtTLP tubby domains with the mouse TUBBY domain revealed a putative PI (4,5) P_2 binding domain in each AtTLP, except AtTLPs 4 and 8. This suggests that AtTLPs 1-3, 4-7, and 8-11may bind to PI (4,5) P_2. It is known that the mouse TUBBY protein is a bipartite transcription regulator. Its tubby domain possesses double-stranded DNA binding activity, and its N-terminal segment seems to modulate transcription (Boggon et al., 1999, Science 286: 2119-2125). In plants, the N-terminal region of TLPs is quite different from that in mammal TLPs as AtTLP9-GAL4 DNA binding domain fusion protein failed to activate transcription from a GAL4 promoter in a heterologous system.

Location and Gene Structure Comparison of the AtTLP Gene Family

Chromosome localizations of each AtTLP genes were determined using Map View (www.arabidopsis.org/servlets/mapper) (Huala et al., 2001, Nucleic Acids Res 29: 102-105). It was found that the genes were not evenly distributed on chromosomes I, II, III, and V. Seven genes (AtTLPs 1, 4, 5, 6, 7, 8, and 10) were located on chromosome I, and two genes (AtTLPs 2 and 3) were located on chromosomes II. The other two, AtTLPs 9 and 11, were located on chromosomes II, III respectively. Although most of the AtTLP genes were located on chromosome I, no local tandem repeats or gene clusters were identified.

By comparing the sequences of the RT-PCR products and the Arabidopsis genome, the corrected exon-intron organizations of the AtTLP genes (except for AtTLP4) were determined. It was found that exon 1 contained the sequences encoding each protein's N-terminal leading sequence, the F-box, and a nine-residue spacer between the F-box and tubby domain. This result indicated that the genes might have arisen from the same ancestral gene. The sequence encoding the C-terminal tubby domain was found to distribute in exons interrupted by 2 or 3 introns. On the basis of the exon and intron composition, the AtTLP genes were classified into three groups. Each gene of the first group (AtTLPs 1, 2, 5, 6, 7, and 10) contains three introns. Each of the second group, AtTLPs 3, 9 and 11, contains an additional intron in the region encoding the C-terminal part of the tubby domain. The third and the most distinct group (AtTLPs 4 and 8) contain 5 and 8 introns, respectively.

Expression of AtTLP Genes

A coupled RT-PCR based assay was conducted to determine the expression pattern of AtTLP genes. Total RNA was isolated from roots, main and lateral stems, rosette leaves, flower clusters, and green siliques of 42-days-old soil-grown Arabidopsis. For each gene, a pair of gene-specific primers was chosen, and PCR amplifications were carried out using 15 ng of first strand cDNA synthesized as described above. Primers of ubiquitin gene, UBQ10, (5'-ATTTCTCAAAATCTTAAAAACTT-3' and 5'-TGATAGTTTTCC CAGTCAAC-3') were used to amplify ubiquitin, which served as an internal loading standard (Norris et al., 1993, Plant Mol. Biol. 21: 895-906).

The results showed that AtTLPs 1, 2, 3, 6, 7, 9, 10 and 11 were expressed in all organs tested, with slight variations in mRNA accumulation. In contrast, AtTLPs 5 and 8 were primarily expressed in the root, flower, and silique. The organ-specific expressions of AtTLPs 5 and 8 indicate their specific roles in particular organs.

Although the expression of AtTLP1, 2, 3, 6, 7, 9, 10 and 11 is present in all tissues tested, the possibility that these genes are expressed with cell type specificity could not be excluded. It is possible that differential expression of these AtTLP genes could only be observed when internal developmental programming was altered or specific environmental stimuli were applied to the plants. To test this hypothesis, the public Arabidopsis Functional Genomics Consortium (AFGC) microarray expression database (the Stanford Microarray Database, genome-www5.stanford.edu/MicroArray/SMD/) (Wu et al., 2001, Plant Physiol Biochem 39: 917-926) was searched. Twofold expression was used as the difference cutoff. Based on the search, the expression profiles of DNA fragments corresponding to AtTLP2, 7, 9 and 10 were summarized in Table 2 below.

Table 3 Microarray analysis of *AtTLP* genes expression

Experiment	Channel 1 Description	Channel 2 Description	Ch2/Ch1 Normalized (Mean)[b, c]			
			AtTLP2	*AtTLP7*	*AtTLP9*	*AtTLP10*
Hormone Effect						
Auxin Response	msg seedlings, untreated	msg seedlings, 10 uM IAA for 30 min	0.32	2.21		
Auxin Induction	Mock-treated Columbia roots	NAA-treated Columbia roots		0.46		2.22
Cytokinin response	Control	15 min cytokinin treatment	2.19			
Abscisic acid Insensitive 1	Wild type control	*Abscisic acid insensitive 1* mutant	0.49	2.6	0.35	
***edr1* Mutant**	Wild type leaves	*edr1* mutant leaves	2.33			
Downstream genes of *KN1*	Control	Overexpression of KN1-GR in Columbia-0 background	0.43	2.78		4.73
Stress						
Effects of Elevated atmospheric CO_2	Columbia leaves 360ppm CO_2	Columbia leaves 1000ppm CO_2	0.33			
Genes involved in chilling tolerance	Cold treated Columbia wild type tissue	Cold treated *cls8* mutant tissue	0.22		0.15	
Genes involved in potassium nutrition	[K+]=120uM	[K+]=2mM		0.34		0.2
Cadmium	Control	10uM cadmium treated plant			2.72	
Light Signaling						
Circadian rhythm time = 12,0	Time = 0 hrs	Time = 12 hrs	0.37			
Phototropic stimulation	Seedlings grown in the dark and exposed to 1hr blue light	nph4-2 seedlings grown in the dark and exposed to 1 hr blue light	2.08			
Protein import into chloroplasts: CIA-2	Wild type	*cia-2* (mutant)				3.46
Identification of genes in chlorophyll starvation	WT leaves after exposure to 230uE for 2 days	cch1 leaves after exposure to 230 uE for 2 days				2.02
Stress						
Effects of Elevated Atmospheric CO_2	Columbia leaves 360ppm CO_2	Columbia leaves 1000ppm CO_2	0.33			
Genes involved In chilling tolerance	Cold treated Columbia wild type tissue	Cold treated *cls8* mutant tissue	0.22		0.15	
Genes involved in potassium nutrition	[K+]=120uM	[K+]=2mM		0.34		0.2
Cadmium	Control	10uM cadmium treated plant			2.72	

[a] These data are obtained from http://afgc.stanford.edu/afgc_html/site2.htm

[b] All data are corresponding with fluorescence intensities greater than 500 in both channels and ch2/ch1 normalized ratio $\geqq$ 2.0 or $\leqq$ 0.5

[c] When searching dbEST with blastn, we find *Arabidopsis* EST corresponding to fragments of four *AtTLPs* represented on microarray data generated by AFGC. *AtTLP2* is corresponding to the EST clone 289B10T7 and 173K22T7. *AtTLP7* *AtTLP9* and *AtTLP10* are corresponding to the EST clone 173G1T7, 201E19T7 and F3E6T7, respectively.

The resulted show that factors like hormone fluctuation and environmental stimuli modulate the expression of the four AtTLP genes. As shown in Table 2, the four AtTLP genes

had different responses to treatments of various hormones. AtTLP2 gene expression instantaneously increased more than twofold with cytokinin treatment but decreased to one-third after being treated with IAA. This suggests that Cytokinin and auxin play antagonistic roles in regulating AtTLP2 gene expression.

Another cytokinin-related experiment was aimed at identifying downstream genes of KN1. KN1-like protein is a homeobox transcription factor. Its overexpression upregulates cytokinin production and leads to delayed senescence (Vollbrecht et al., 1991, Nature 350: 241-243). The expression of AtTLP7 and 10 is upregulated in KN1 overexpression transgenic plant while AtTLP2 is downregulated.

The different responses of AtTLPs 7, 2, and 10 to ABA treatment is also worth noticing. In abscisic acid insensitive 1 mutant (Pei et al., 1997, Plant Cell 9: 409-423), the expression of AtTLP2 and AtTLP10 decreases by two to threefold, but AtTLP7 expression increases over twofold. Interestingly, AtTLP2 and AtTLP7 behaved oppositely to auxin treatment and in abscisic acid insensitive 1 mutant and KN1 overexpression transgenic plant. These two AtTLPs therefore may function antagonistically in regulating phytohormone-signaling pathways.

The expression level of AtTLP2 rose in the edr1 (enhanced disease resistance I) mutant leaves. The EDR1 gene encodes a putative MAP kinase similar to CTR1, a negative regulator of ethylene response in Arabidopsis (Frye et al., 2001, Proc. Nat. Acad. Sci. 98: 373-378). The edr1 mutation of Arabidopsis also confers resistance to powdery mildew disease (Frye and Innes, 1998, Plant Cell 10: 947-956). Thus, the regulation of AtTLP2 gene expression may be associated with SA-inducible and ethylene defense mechanism.

Environmental stresses also impose influences on the expression of AtTLP genes. For example, similar to the cold treatment on cls8 mutant, elevated CO_2 level inhibited the expression of AtTLP2. K^+ deficiency augmented the expression of AtTLPs 7 and 10 by threefold and fivefold, respectively. Heavy metal cadmium treatment stimulated the expression of AtTLP9.

In conclusion, the expression data of these four AtTLP genes indicate their involvement in phytohormone and environmental stress signaling.

AtTLP9 Interacts with ASK1 Protein

Homology searches in the public databases reveal that TLPs were also present in multiple plant species, including Lemna paucicostata, Oryza sativa, Cicer arietinum, maize, and Arabidopsis. Unlike animal TLPs having highly diverse N-terminal sequences, plant TLPs had conserved F-box-containing domain. Sequence alignment of the F-box cores from AtTLP, TIR, UFO, COI1 and the human F-box protein Skp2 revealed conserved islands separated by regions with weak homology. Many of the conserved residues correspond with those known to be important for Skp association (Schulman et al., 2000, Nature 408: 381-386 and Zheng et al., 2002, Nature 416: 703-709).

The F-box domain, first found in cyclin-F, interacts with the protein SKP1, which interacts with the Cdc53 (Cullin) proteins, to form a so-called SCF complex. The F-box is involved in recruiting specific proteins (e.g., transcription activators or repressors) and targeting them for ubiquitin-mediated proteolysis by 26S proteosome. Analysis of the *Arabidopsis* genome revealed that *Arabidopsis* had 21 Skp1-like, or ASK, protein, which exhibited different expression patterns. Among them, ASK1 is involved in vegetative growth and reproductive development (Zhao et al., 2003, Plant Physiology 133: 203-217).

To test whether AtTLP could interact with ASK1, AtTLP9 was examined by the yeast two-hybrid analysis. Yeast two-hybrid vectors, pAD-GAL4-2.1 and pBD-GAL4 Cam (Stratagene, La Jolla, CA), were used for C-terminal GAL4 AD and BD fusion constructions, respectively. A 1.1-kb SalI-PstI fragment containing the entire coding region of AtTLP9 was cloned into the SalI-PstI site of the pBD-GAL4 Cam vector. A 480-bp EcoRI-PstI fragment containing the entire coding region of ASK1 (At1g75950) was cloned into the EcoRI-PstI site of the pAD-GAL4-2.1 vector. The yeast strain YRG-2 [MATa ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 gal4–542 gal80–538 LYS2::GAL1UAS–GAL1TATA–HIS3 URA3::(GAL43 × 17mer)-CYC1TATA–lacZ] was co-transformed with the two vectors. The Y2H analysis was performed according to the manufacturer's recommendations (Stratagene). The result suggested that AtTLP9 physically interacts with ASK1 to form SCF complex and acts as a factor for substrate recognition in the ubiquitin-mediated proteolysis.

Attlp9 Null Mutants and Overexpression Lines

ATLP9 was analyzed to investigate for it in vivo functions. Both loss-of-function and overexpression approaches were taken to address its biological roles.

To identify attlp9 T-DNA insertion mutant, AtTLP9 (At3g06380) was used to search the T-DNA Express database at http://signal.salk.edu/cgi-bin/tdnaexpress. Two attlp9 T-DNA insertion mutants (ABRC seed stock SALK_016678 and 051138) were identified and designated as attlp9-1 and attlp9-2. T_3 seeds of attlp9-1 and attlp9-2 were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus). The position of the T-DNA within the AtTLP9 gene was re-confirmed by sequencing a PCR-amplified fragment amplified by primer pairs corresponding to the T-DNA left borders and the AtTLP9 gene specific primer. The following primer pairs were used for attlp9-1 and attlp9-2 specific amplification,

attlp9-1: N1, 5' -ATGACGTTCCGAAGTTTACTC- 3';

LBa1, 5' -TGGTTCACGTAGTGGGCCATC- 3';

attlp9-2: C1, 5' -TTATTCACAGGCAATTCTGGT- 3'; and

LBa1, 5' -TGGTTCACGTAGTGGGCCATC- 3'.

It was found that Attlp9-1 had a T-DNA insertion in the coding sequence at codon 705, whereas attlp9-2 had an insertion in the 5' distal region of this gene. The T-DNA insertion site of attlp9-1 was identical to that originally described in the T-DNA Express database. However, the T-DNA insertion site of attlp9-2 was in the promoter region instead of exon1 as predicted in the database (the latter is supported by a potential full length cDNA corresponding to At3g06380 generated in RIKEN, accession number BT004092).

Southern blot was conducted with the nptII marker gene to determine the T-DNA insertion number in attlp9-1 and 9-2 knockout mutants. It was found that one and three T-DNA insertion events in the T_4 attlp9-1and attlp9-2 T-DNA insertion mutants, respectively.

The T-DNAs in attlp9-1 and attlp9-2 carried a gene leading to resistance to kanamycin. Homozygous analyses of attlp9-1 and attlp9-2 plants were carried out by kanamycin selection and PCR based method. RT-PCR analyses of T_4 homozygous of attlp9-1 and attlp9-2 plants indicated that attlp9-1 was a null allele, whereas attlp9-2 was somewhat leaky. For the phenotype investigation, attlp9-1 and attlp9-2 T_4 homozygous lines were used for detailed analysis.

Transgenic plants with overexpressing AtTLP9 were generated. An XbaI-SmaI fragment of AtTLP9 was inserted into an XbaI-SmaI site of the pBI121 Ti-vector (Clontech) to generate a 35S:: AtTLP9 sense construct. The XbaI-SmaI fragment contained the entire AtTLP9 coding region and was under the control of the 35S promoter of cauliflower mosaic virus. The constructs were introduced into Agrobacterium strain LBA4404 by electroporation and transformed into wild-type plants by the floral dip method (Clough et al., 1998, Plant J 16: 735-743). 38 independent transgenic lines (T_0 generation) were obtained. Among them, seven independent homozygous lines from the T_3 sense transgenic plants were analyzed for the AtTLP9 expression. Each of these lines contained a single copy of the transgene. Two independent transgenic lines (S13-2 and S16-1) showed dramatic increases in the AtTLP9 transcript levels and were further analyzed. A number of control transgenic lines were generated by transforming with Agrobacterium with PBI121 vector alone.

The wild type Arabidopsis thaliana ecotype Columbia-0 (Col-0) and mutant abi4-1 (obtained from Dr Wan-Hsing Cheng, Institute of Botany, Academia Sinica Taipei) were used. The phenotypes of abi4-1 were confirmed as described (Söderman et al., 2000, Plant Physiol. 124: 1752-1765) prior to use.

All seeds of the above-described lines were surface sterilized with 70% ethanol for 30 s and then with 6% household bleach for 5 min before being washed five times with sterile water. For aseptic growth, they were plated on solid medium containing Murashige and Skoog salts (Invitrogen), vitamins (Duchefa), 0.7% phytoagar (Invitrogen), and 1% sucrose and transferred to a tissue culture room. For soil growth, seedlings were transferred into individual pots 8-10 days after germination and maintained in the growth chamber. Plants were grown at 22°C under a 16-hr-light/8-hr-dark photoperiod aseptically or on soil.

The general development and growth phenotypes of the attlp9-1 and attlp9-2 knockout plants appear to be similar to those of the wild type plants. However, when seeds were plated on nutrient agar media, the germination time of mutant attlp9-1 and attlp9-2 seeds was advanced several hours compared with that of the wild type plants, whereas the selected sense line seeds (i.e., S13-2 and S16-1) germinated later than vector control seeds. It was found that 50% of the wild type seeds geminated after about 37 hour after plating. In contrast, 50% of the attlp9-1 and attlp9-2 knockout seeds geminated at hours 26-28 after plating, and 50% of the S13-2 and S16-1 seeds geminated around hours 40-42 hours after plating.

Effect of ABA on Seed Germination of Attlp9 Mutants and Overexpression Lines

It is known that seed germination is the outcome of an integration of many signals coordinated by the interactions of stage-specific developmental regulators and the competing effects of hormonal signals (Finkelstein et al., 2002, Curr. Opin. Plant. Biol. 5: 26-32). The most critical hormone promoting embryo maturation and preventing germination is ABA.

To determine whether the transgenic plants display altered ABA responses, the above-described lines were germinated on media containing various concentrations of ABA. Seeds collected at the same or similar times were used. After surface-sterilization, sterile seeds were suspended in 0.15% agarose, and kept in the dark at 4°C for 3 days to break residual dormancy. The seeds were then plated on agar plates in six replicates containing no ABA or 0.25, 0.5, 0.75, or 1.0μM ABA in 12-cm plastic petri dishes. Each agar plate was divided into seven sections, and 50 seeds of WT and AtTLP9 transgenic seeds were plated on each section. A seed was regarded as germinated when the radicle protruded through the seed coat.

In the presence of 1μM ABA, the germination of sense lines seeds was further delayed and the germination rate was reduced to less than 10%. In contrast, the germination rate of attlp9-1 and attlp9-2 mutant seeds nearly reached 50%, and about 30% of wild-type seeds were able to germinate in the presence of 1μM ABA. These results suggest that the disruption of the AtTLP9 gene affects the sensitivity of seeds to exogenous ABA.

In addition to reducing seed germination rate, ABA also inhibited the growth and the greening process in cotyledons of the sense transgenic lines. In MS agar medium containing 1μM ABA and 1 % sucrose, 90% of the 10-d-old seedlings showed developmental arrest although the radicles of most sense lines seeds emerged. In contrast, under the same conditions, attlp9-1 and attlp9-2 plants continued to grow and about 45% of the seedlings continued to develop true leaves, although at slower rates than abi4-1 mutant does. These results indicate that the alteration of AtTLP9 modulate plant's sensitivity to ABA during seed germination and early seedling development.

AtTLP9 Expression is Transiently Up-regulated During Imbibition of Seeds

Real-time PCR experiments were conducted to quantify AtTLP9 transcript levels at seed maturation, seed germination, and early development stage. UBQ10 was used as the endogenous

control (Norris et al., 1993, Plant. Mol. Biol. 21: 895-906). Primers were designed using Primer Express 1.0 software (Applied Biosystems). The primers used were:

AtTLP9 forward primer, 5'-TAGGCCACACCGTGTAGTTCA-3';

AtTLP9 reverse primer, 5'-CGTCAACAGTCTCAACCCTAATCA-3';

UBQ10 forward primer, 5'- AGAAGTTCAATGTTTCGTTTCATGTAA -3'; and

UBQ10 reverse primer, 5'- GAACGGAAACATAGTAGAACACTTATTCA -3'.

The real-time PCR was performed in a 50 μL reaction mixture containing 500 ng first strand cDNA, 2.5 μM each primers and 1×SYBR Green PCR Master Mix (Applied Biosystems). PCR cycling was 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 15 sec at 95°C/1 min at 60°C. The UBQ10 mRNA quantity was set at '1' and AtTLP9 expression was determined relative to control samples. Threshold cycles were determined by Sequence Detection System V. 1.7a software (Applied Biosystems). The products were quantified by the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Scoresby, Victoria, Australia).

Seed germination is divided into three phases: imbibition, increased metabolic activity, and initiation of growth (Bewley. 1997, Plant Cell 9: 1055-1066). It was found that during seed maturation and seed imbibition at 4°C for 72h, AtTLP9 transcripts remained at a relatively low level. When the seeds were transferred to 22°C for further incubation, the levels rose after 8 h, peaked at 16h, and fell rapidly after 24h when the radicle emerged. The AtTLP9 transcripts were barely detectable afterwards. The transient expression of AtTLP9 indicated that AtTLP9 functions at stage II of seed germination as a checkpoint before radicle protrusion.

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the scope of the following claims.